

Supplementary Information

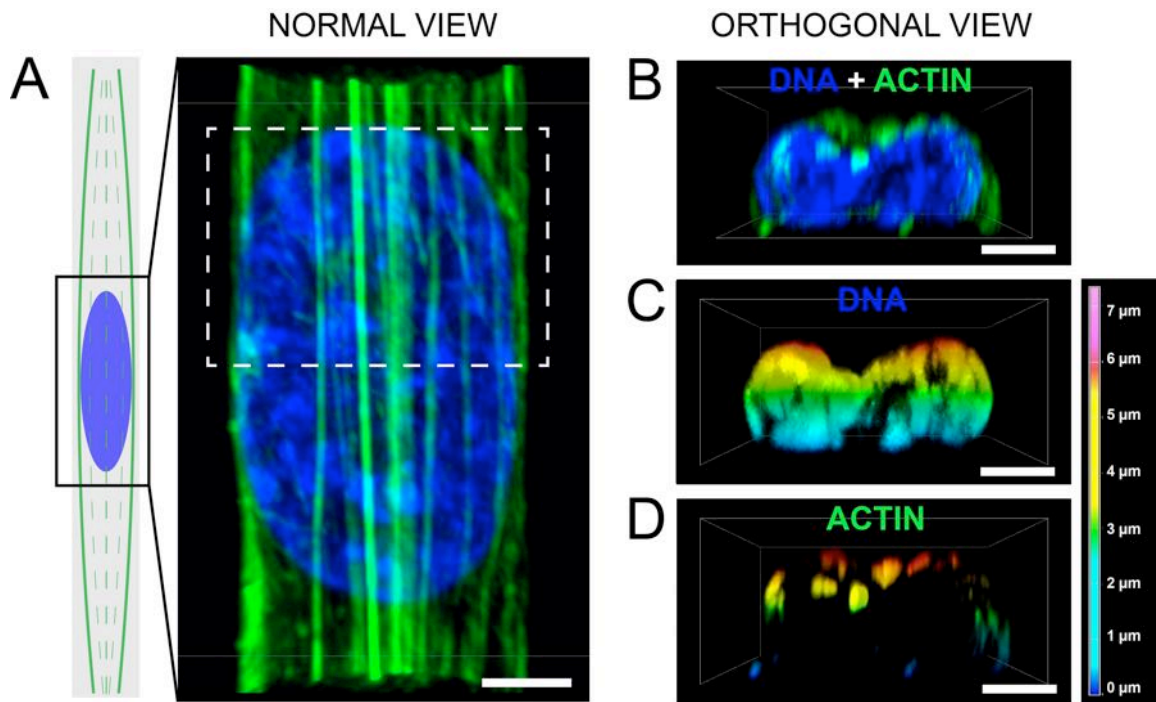
Super-resolution microscopy reveals a LINC complex recruitment at nuclear indentation sites

Marie Versaevel¹, Jean-Baptiste Braquenier², Maryam Riaz¹,
Thomas Grevesse¹, Joséphine Lantoiné¹
& Sylvain Gabriele^{1*}

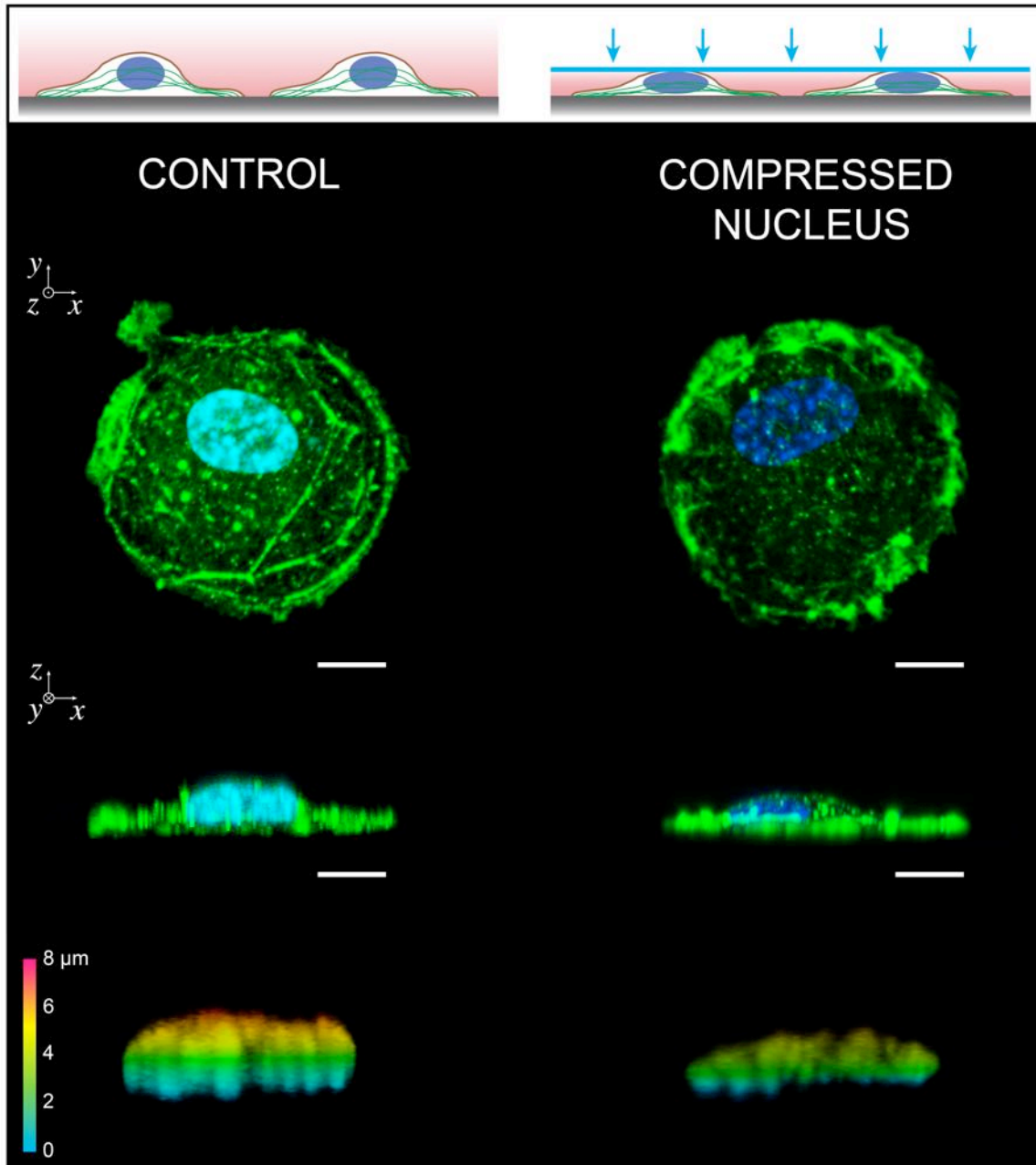
¹ Mechanobiology & Soft Matter Group, Interfaces and Complex Fluids Laboratory, Research Institute for Biosciences, CIRMAP, University of Mons, 20, Place du Parc, B-7000 Mons, Belgium

² Nikon Belux Instruments, 50 B Avenue du Bourget, B-1130 Brussels, Belgium.

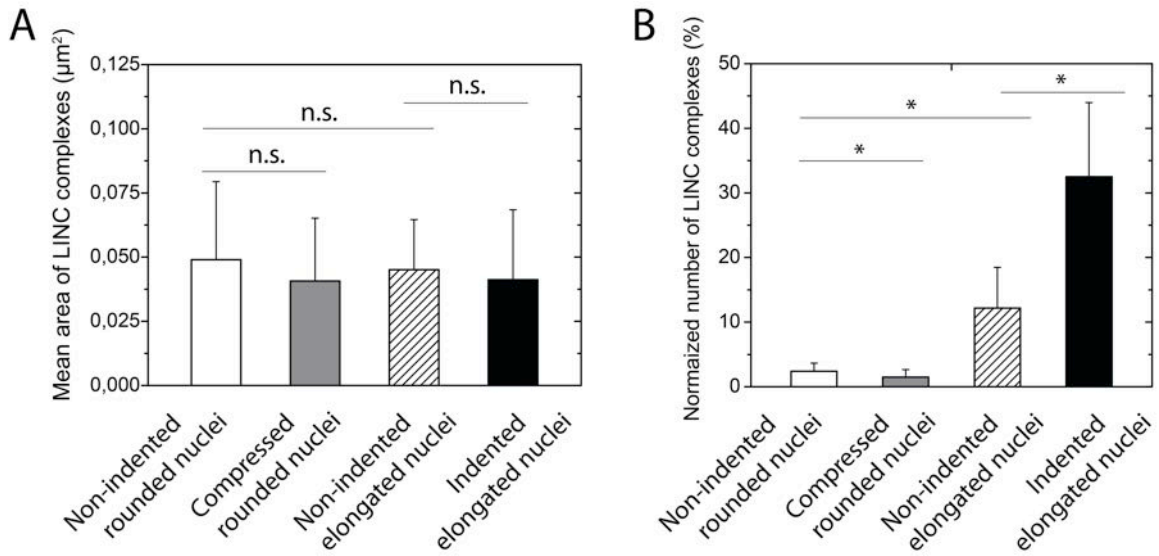
* Correspondence and requests should be addressed to S.G.
(sylvain.gabriele@umons.ac.be)



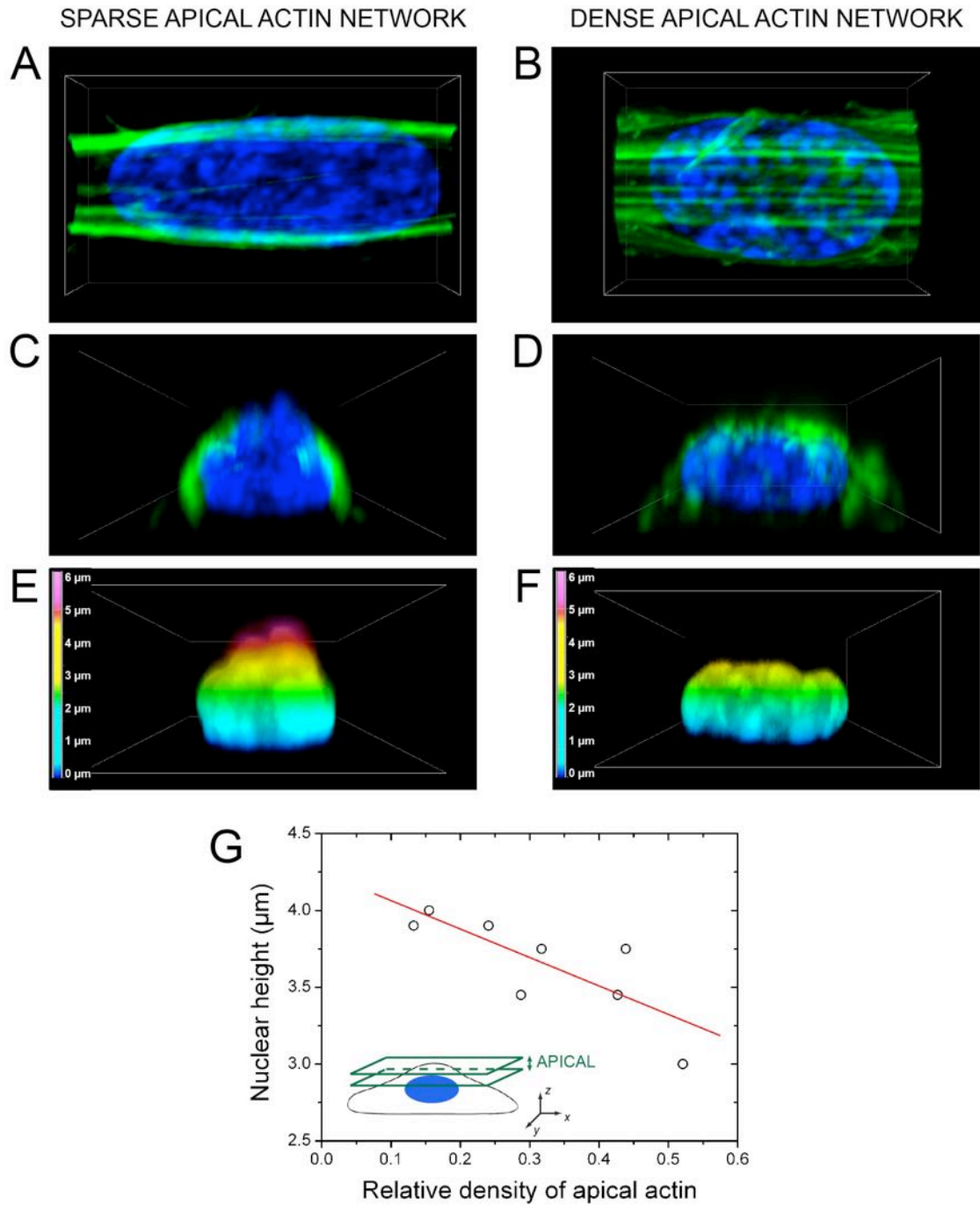
Supplementary Figure 1 – (A) Schematic representation and normal view (XY) of an elongated nucleus surrounded by actin filaments. The confocal image shows the spatial organization of the DNA (in blue) and the actin cytoskeleton (in green) of an endothelial cell spread on a rectangular micropattern. 3D confocal cross-section views (XZ) of (B) the nucleus (in blue) and the actin cytoskeleton (in green), (C) a Z-depth coding of the nucleus (Dapi staining) and (D) a Z-depth coding of the actin cytoskeleton. For a better visualization, 3D confocal cross-section views presented in (B), (C) and (D) have been obtained from the middle zone of the nucleus, as depicted by the dashed rectangle in (A). Scale bars represent 3 μm .



Supplementary Figure 2 – Orthogonal views (XY) and cross-sections (XZ) views of the nucleus (blue) and the actin cytoskeleton (green) of endothelial cells observed by confocal microscopy. The left column correspond to a typical single endothelial cell plated on a circular FN-coated micropattern and the right column to a nuclear compressive experiment performed on a circular-shaped endothelial cell. A Z-depth coding of the nucleus (Dapi staining) obtained both conditions shows the flattening of the nucleus under normal compressive forces.



Supplementary Figure 3 – (A) Evolution of the mean nuclear are of LINC complexes for non-indented rounded nuclei (white bar), compressed rounded nuclei (grey bar), non-indented elongated nuclei (hashed bar) and indented nuclei (black bar). (B) Evolution of the normalized number of LINC complexes for non-indented rounded nuclei (white bar), compressed rounded nuclei (grey bar), non-indented elongated nuclei (hashed bar) and indented nuclei (black bar). Datas are mean \pm SD. * $p < 0.05$ and n.s.: not significant using unpaired Student's test.



Supplementary Figure 4 – Orthogonal views of the nucleus (blue) and the actin cytoskeleton (green) of endothelial cells spread on a rectangular micropattern (1:10 aspect ratio) with (A) few apical actin stress fibers and (B) a dense apical network of parallel actin fibers. (C) and (D) are cross-sections views (XZ) obtained from (A) and (B) respectively. (E) and (F) are Z-depth coding of the nucleus (Dapi staining) obtained from (C) and (D), respectively. A dense network of apical stress fibers (B and D) leads to a reduced nuclear height (F). (G) The nuclear height of endothelial cells

(n=8) spread on rectangular micropatterns (1:10 aspect ratio) decreases linearly with the relative density of apical actin fibers.

Supplementary Movie 1 – Confocal Z-stack reconstruction of the lamina indentations observed in a deformed nucleus. The scale bar corresponds to 2 μm .

Supplementary Movie 2 – Cross-sectional reconstruction (XZ) from a 3D confocal image of the nucleus (DNA in blue), the actin cytoskeleton (in green) and the lamin A/C (red) of a single endothelial cell spread on a rectangular micropattern (1:10 aspect ratio). The box size (xz) corresponds to 14x9 μm .

Supplementary Movie 3 – 3D reconstruction showing the presence of deep indentations in an elongated nucleus stained for DAPI and observed by confocal microscopy. A box side corresponds to 55 μm .

Supplementary Movie 4 – 3D reconstruction of an elongated nucleus stained for DAPI and observed by confocal microscopy. A box side corresponds to 55 μm . The box size (xz) corresponds to 22x31 μm .

Supplementary Movie 5 – Sequence of the relaxation of the nucleus of a single endothelial cell spread on a rectangular micropattern (1:10 aspect ratio) and treated with accutase. The normal view of the nuclear relaxation was obtained in epifluorescence mode with a H2B-GFP labeling. The scale bar represents 5 μm and the movie is in real time.